H-Y minor histocompatibility antigens and the development of chronic graft versus host disease (cGVHD) after allogeneic hematopoietic stem cell transplantation (HSCT). These findings suggest that donor B cells play a role in the development of cGVHD and clinical trials utilizing anti-CD20 monoclonal antibody, rituximab, have demonstrated clinical improvement in some patients with active resistant cGVHD. B cell activating factor, BAFF, is a member of the TNF cytokine family that binds to one of three cell surface receptors on B cells. Activation of these B cell receptors by antigen and BAFF results in rapid induction of proliferation and differentiation of germinal center B cells into either Ig-secreting cells or memory B cells. The finding that elevated BAFF levels in patients with autoimmune diseases are associated with disease severity and autoantibody production has led to clinical trials using anti-BAFF monoclonal antibody in these diseases. To test whether BAFF contributes to the activation of B cells in patients with cGVHD, we employed a sandwich ELISA to measure soluble BAFF in plasma from 27 patients after allogeneic HSCT. Mean BAFF levels for extensive (n = 8), limited (n = 8), resolved (n = 6) or no (n = 5) cGVHD groups were: 7.3 ng/ml, 5.37 ng/ml, 3.92 ng/ml and 2.02 ng/ml, respectively. The differences between the extensive or limited groups versus the no cGVHD group were statistically significant (each $P = .03$) suggesting that BAFF levels corresponded with cGVHD activity. B cell subsets in peripheral blood were also examined by flow cytometry to identify circulating memory B cells. Of four cGVHD patients analyzed thus far, individuals with active disease and high BAFF levels also had high numbers of CD27+ memory B cells in peripheral blood compared to patients with resolved cGVHD and low BAFF levels. Ongoing prospective, serial assessment of BAFF levels and B cell phenotype in larger numbers of patients will extend these results. We propose that BAFF may serve as a new biomarker for cGVHD, specifically identifying patients with active disease. If further studies identify a pathologic role for increased BAFF in cGVHD, anti-BAFF monoclonal antibodies may provide a new therapeutic approach for this serious complication of allogeneic HSCT.

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TREATMENT OF MURINE ACUTE GVHD WITH THE NOVEL PRO-APOPTOTIC BENZODIAZEPINE BZ-423

Gatza, E.[1], Clouthier, S.[1], Rogers, C.[1], Liu, C.[2], Reddy, P.[1], Glick, G.D.[1], Ferrara, J.L.M.[1] 1. University of Michigan Comprehensive Cancer Center, Ann Arbor, MI; 2. University of Florida College of Medicine, Gainesville, FL.

Bz-423 is a novel 1,4-benzodiazepine with both cytotoxic and cytostatic effects on activated lymphocytes. Bz-423 has been shown to suppress lupus in murine models by eliminating pathogenic lymphocytes without altering normal immune function or overt toxicities. We hypothesized that Bz-423 might enhance the apoptotic deletion of alloreactive donor T cells and therefore reduce graft-versus-host disease (GVHD). We first investigated the effect of Bz-423 on activated T cells in vitro. A 40 μM concentration of Bz-423 induced apoptotic death in >90% of both $CD4^+$ and $CD8^+$ T lymphocytes stimulated with allogeneic dendritic cells (DC) or ConA in the presence of syngeneic DC. Purified resting T cells were significantly less susceptible to Bz-423-induced apoptosis (data not shown). We next examined the effect of Bz-423 in vivo utilizing a well-characterized (Balb/c→B6) murine GVHD model. B6 recipient mice were conditioned with 11 Gy total body irradiation and injected with 5.0×10^6 bone marrow and $4\text{-}5.0 \times 10^6$ purified T cells from either syngeneic (B6) or allogeneic (Balb/c) donors. In order to model the administration of Bz-423 as a treatment strategy rather than a prevention strategy, animals were not treated for the first seven days after BMT. Beginning 7 days following BMT and continuing for the remainder of the experiment, Bz-423 was administered i.p. at a dose of 60 mg/kg three times weekly. As shown in the table below, treatment with Bz-423 resulted in significantly better day +60 survival ($P = .001$) and reduced clinical GVHD scores ($P < .02$) among allogeneic BMT recipients. Bz-423 also reduced GVH-associated immunodeficiency ($P = .02$) and decreased liver and gut histopathology of GVHD ($P = .01$). Delay of the onset of treatment to day 14 post-BMT also resulted in a significant survival benefit ($P = .03$). These data demonstrate that Bz-423 can reverse acute GVHD and induce selective apoptosis of alloreactive donor T cells in this model. Pro-apoptotic benzodiazepines may provide a strategy for the treatment of acute GVHD (Table 1).

Table 1.

	Syn + Bz	Allo + vehicle	Allo + Bz	P Value[1]
Treatment onset: day +7				
Survival (day 60)	100% (n = 9)	8% (n = 13)	59% (n = 19)	.001
GVHD clinical score (day 60)[2]	1.5 ± 0.2	6.5 ± 0.0	3.1 ± 0.4	<.02
Pathology (day 74)[3]				
Liver	1.3 ± 0.6	13.7 ± 1.5	8.3 ± 2.5	.01
Intestine	3.0 ± 2.0	21.3 ± 6.0	10.0 ± 0.8	.01
Splenic reconstitution (day 74)[3]				
T cells ($\times 10^6$)	20.6 ± 1.8	1.4 ± 0.02	3.6 ± 0.4	.02
B cells ($\times 10^6$)	41.6 ± 12.6	2.5 ± 0.7	21.2 ± 15.6	.02
Treatment onset: day +14				
Survival (day 60)	100% (n = 3)	20% (n = 5)	71% (n = 6)	.03

[1]Calculated at the 95% confidence level, Allo + vehicle vs Allo + Bz-423; [2]reported as mean clinical score ± SE; [3]reported as mean value ± SD.

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OPPOSING EFFECTS OF ICOS ON GRAFT-VERSUS-HOST DISEASE MEDIATED BY CD4 AND CD8 T CELLS

Yu, X.-Z.[1], Liang, Y.[1], Roza, N.I.[2], Anasetti, C.[1], Dong, C.[2] 1. H. Lee Moffitt Cancer Center, Tampa, FL; 2. M.D. Anderson Cancer Center, Houston, TX.

Inducible costimulatory molecule (ICOS), a CD28 family member expressed on activated CD4+ and CD8+ T cells, plays important roles in T cell activation and effector function. Here we studied the role of ICOS in graft-versus-host disease (GVHD) mediated by CD4+ or CD8+ T cells in allogeneic bone marrow transplantation (BMT). In comparison of wild type (WT) and ICOS-deficient T cells, we found that recipients of ICOS−/− CD4+ T cells exhibited significantly less GVHD morbidity and mortality. ICOS−/− CD4+ T cells had no defect in expansion, but expressed significantly less FasL and produced significantly lower levels of IFN-γ and TNF-α. Thus, ICOS−/− CD4+ T cells were impaired in effector functions that lead to GVHD. In contrast, recipients of ICOS−/− CD8+ T cells exhibited significantly enhanced GVHD morbidity and mortality. In the absence of ICOS signaling, either using ICOS-deficient donors or ICOS-ligand deficient recipients, the levels of expansion and Tc1 cytokine production of CD8+ T cells were significantly increased. The level of expansion was inversely correlated with the level of apoptosis, suggesting that increased ability of ICOS−/− CD8+ T cells to induce GVHD was resulted from the enhanced survival and expansion of those cells. Our findings indicate that ICOS has paradoxical effects on the regulation of alloreactive CD4+ and CD8+ T cells in GVHD.

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HIGHER CLEARANCE RATES OF MYCOPHENOLATE MOFETIL (MMF) IN PEDIATRIC ALLOGENEIC STEM CELL TRANSPLANT (AlloSCT) RECIPIENTS

Militano, O.[1], Roache, K.[2], Shaw, L.M.[3], Figurski, M.[3], Satwani, P.[2], Ayello, J.[2], Roman, E.[2], Del Toro, G.[2], Bradley, B.[2], George, D.[2],

Garvin, J.H.[2], Bhatia, M.[2], Wolownik, K.[2], Foley, S.[2], Hawks, R.[2], Cairo, M.S.[2,4,5] 1. Department of Pharmacy, New York Presbyterian Hospital, New York, NY; 2. Department of Pediatrics, Columbia University, New York, NY; 3. Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA; 4. Department of Pathology, Columbia University, New York, NY; 5. Department of Laboratory Medicine, Columbia University, New York, NY.

To date there are no published data on pharmacokinetics (PK) of MMF in children undergoing AlloSCT. The objective of this study is to evaluate effects of age on the PK of MMF in pediatric AlloSCT pts. From 1/04-9/05 we enrolled 26 pediatric AlloSCT with 23 being evaluable: mean age 7.6 yrs; wt 31.6 kg; M:F = 11:12; NBL PR (n = 3), SCD (n = 2), AML (CR1 [n = 3], CR2 [n = 1], CR3 [n = 1], relapsed/induction failure [n = 2]), SAA (n = 4), CML CP (n = 1), ALL (CR1 [n = 1], CR2 [n = 2], CR3 [n = 1]), HD CR2 (n = 1), ALCL refractory (n = 1); donor sources: MFD (6/6 PBSC [n = 6], 6/6 BM [n = 2], 5/6 PBSC [n = 2]), 6/6 related CB (n = 1), UCB (6/6 [n = 2], 5/6 [n = 2], 4/6 [n = 7]), and 8/10 MUD PBSC (n = 1). Cohort 1 (<6 yrs) (n = 8); 2 (6-12 yrs of age) (n = 8); 3 (12-16 yrs) (n = 7). GVHD prophylaxis included tacrolimus (starting on day −1 or 1st day of conditioning to maintain concentrations 5-20 ng/mL) and MMF (900 mg/m^2/dose IV Q6h starting on day +1, then converted to PO [same dose] after day +14). Serum samples for MPA assay were drawn on day +1, +7, and +14 at hour 0, 0.5, 1, 2, 3, 4, and 6 post-dose. MPA plasma concentrations were determined by reverse-phase HPLC. MMF dose was adjusted to maintain MPA trough 1-3.5 mg/L. The mean CD34$^+$ cell dose/kg = 25.8×10^5, TNC dose/kg = 48×10^7. Time to neutrophil (ANC ≥500/mm^3 × 2 d) and platelet engraftment (untransfused count ≥20K × 7 d) were 22 d and 39 d. The mean f/u was 278 d. Mean MPA PK on day +14: Cmax = 12.4 mg/L, total MPA trough = 0.9 mg/L, $AUC_{0\text{-}12}$ = 32.7 mg · hr/L, Css = 2.7 mg/L, $T_{1/2}$ = 1.5 h , Vss = 1.8 L/kg, and CL = 1.4 L/kg/h at a mean MMF dose of 1080 mg/m^2 IV Q6h. The breakdown of age cohorts is shown in Table 1. Incidence of GI adverse events attributable to MMF was 65% (nausea/vomiting [n = 12], diarrhea [n = 7], abdominal pain [n = 3], pneumatosis intestinalis [n = 1], colitis [n = 1]). Incidence of grade II-IV aGVHD was 59% (13/22 evaluable pts) and cGVHD was 26.3% (5/19 evaluable pts). Kaplan-Meier probability of 1 year OS was 70.4% (CI: 49.7-91.1%). In comparison to MMF PK in adult AlloSCT pts receiving cyclosporine/MMF (Nash et al, *Biol Blood Marrow Transplant*, 2005), children have significantly higher MMF clearance rates (1.4 vs 0.54 L/kg/h). MMF doses >3-fold higher than those used in pediatric SOT recipients were required to achieve $AUC_{0\text{-}12}$ = 30-60 mg · h/L. Short half-life (1.5 hrs) and rapid clearance of MMF in pediatric AlloSCT pts may be related to a lack of enterohepatic recycling and enhanced UDP-glucuronosyltransferase activity (Table 1).

Table 1. *Mean Age-Related IV MMF PK on Day +14*

Age Group	Cmax (mg/L)	Total MPA Trough (mg/L)	Free MPA Trough (ng/mL)	AUC 0–12 (mg · hr/L)	Total Css (mg/L)	T1/2 (hr)	CL (L/kg/hr)
<6 y.o. (n = 6)	12.2	1.0	18.7	34.0	2.8	1.8	1.6
6–12 y.o. (n = 5)	11.8	0.4	14.8	29.5	2.5	1.1	1.4
12–16 y.o. (n = 2)	14.4	1.4	17.9	39.9	3.3	1.5	0.6

Cmax = peak concentration; MPA = mycophenolic acid; AUC = area under the curve; Css = steady state concentration; T1/2 = half-life; CL = clearance.

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THE ROLE OF MHC CLASS II IN CD4$^+$CD25$^+$ T CELL-MEDIATED FACILITATION OF ALLOGENEIC HEMATOPOIETIC ENGRAFTMENT AND SUPPRESSION OF GVHD

Hanash, A.M., Jones, A., Levy, R.B. University of Miami Miller School of Medicine, Miami, FL.

In recent years there has been significant interest in the potential of CD4$^+$CD25$^+$ regulatory T cells to suppress GVHD and promote allogeneic engraftment. In a MHC-mismatched model utilizing C57BL/6 (B6) T cell-depleted bone marrow and sublethally conditioned (7.0 Gy TBI) BALB/c recipients, we have demonstrated that co-transplanted donor CD4$^+$CD25$^+$ T cells are capable of supporting multi-potential and lineage-committed donor progenitor activity as well as long-term chimerism and tolerance. Little is currently understood regarding the antigen recognition initiating regulatory cell function during hematopoietic transplantation, and we have employed our *in vivo* model toward elucidating the antigenic requirements involved in the initial promotion of allogeneic engraftment. Transplanting BALB/c × B6 F1 CD4$^+$CD25$^+$ T cells (1×10^6) with B6 marrow (2×10^6) significantly increased B6 CFU-GM in BALB/c recipients seven days post-BMT ($P < .001$ vs. BM alone), demonstrating that donor CD4$^+$CD25$^+$ T cells did not require alloreactivity to support hematopoietic progenitors. Furthermore, B6 CD4$^+$CD25$^+$ T cells failed to augment MHC-disparate C3H/HeJ CFU-GM in BALB/c recipients ($P > .05$ vs. BM alone), suggesting that donor CD4$^+$CD25$^+$ T cells might require recognition of syngeneic MHC for progenitor support. Indeed, augmentation of donor CFU-GM was abrogated when B6 CD4$^+$CD25$^+$ T cells were co-transplanted with B6-MHC class II$^{-/-}$ marrow into BALB/c recipients ($P > .05$ vs. BM alone). CD4$^+$CD25$^+$ T cell-mediated augmentation of donor chimerism two months post-BMT was also abrogated by co-transplantation with MHC II$^{-/-}$ marrow. In order to compare the role of MHC class II in progenitor support vs. suppression of GVHD, a model of CD8-mediated GVHD was utilized: co-transplanted BALB/c CD4$^+$CD25$^+$ T cells were able to prevent the GVHD mediated by highly purified BALB/c CD8 T cells in B6-wt recipients, but failed to control the GVH reaction that occurred in B6-MHC II$^{-/-}$ recipients. Therefore, while donor CD4$^+$CD25$^+$ T cells required co-transplantation with syngeneic MHC II to support donor hematopoietic progenitors, allogeneic recipient MHC II was required for suppression of GVHD. In conclusion, donor CD4$^+$CD25$^+$ T cells capable of promoting long-term engraftment and tolerance may have distinct antigenic requirements from those responsible for GVHD suppression, and clinical protocols for allogeneic transplantation involving CD4$^+$CD25$^+$ T cells should account for their distinct antigenic requirements.

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ROLE OF IL-2, IL-7, AND IL-15 IN ALLOGENEIC GRAFT-VS-LEUKEMIA AGAINST ACUTE LYMPHOBLASTIC LEUKEMIA IN A NOD/scid CHIMERIC MURINE MODEL

Cipkala, D.A., Hendey, L., Boyer, M.W. Columbus Childrens Research Institute, Columbus, OH.

We studied the GVL effects of human alloreactive CTL against ALL in a chimeric NOD/scid mouse model. CTL were generated from random blood donor PBMCs stimulated with the 697 human ALL cell line and supplemented with IL-2, −7, or −15. CD8 positive T cells comprised the majority of the cultures in each group: 46% for IL-2, 52% for IL-7, and 45% for IL-15 cultured CTL (n = 13). CTL grown in each cytokine resulted in similar in vitro cytotoxicity: IL-2 41.3%, IL-7 37.7%, IL-15 45.3%, n = 12-15, and had statistically similar intracellular perforin and granzyme-B expression. IL-7 and IL-15 CTL had statistically higher bcl-2 levels than IL-2 CTL suggesting better anti-apoptosis and survival potential. NOD/scid mice were injected with 697 ALL cells followed by 5×10^6 CTL. Mice were sacrificed seven days following CTL injection and residual leukemia was measured in the bone marrow and spleen via flow cytometry. There were two groups of experiments with different degrees of leukemia engraftment seen at day 21. In one group (low engraftment) mice not receiving CTL had a baseline leukemia burden of 2.01% and 0.15% in the bone marrow and spleen, respectively (n = 15). Mice treated with IL-15 cultured CTL had a reduction in tumor burden to 0.2% (n = 13, $P = .01$) and 0.05% (n = 13, $P = .01$) in bone marrow and spleen, respectively. Those treated with IL-2 or IL-7 cultured CTL showed no significant difference in leukemia burden